Genomic DNA from forensic samples

User manual

NucleoSpin® DNA Trace

June 2014 / Rev. 07

MACHEREY-NAGEL
www.mn-net.com

Genomic DNA from forensic samples

Protocol-at-a-glance (Rev. 07)

Funnel

NucleoSpin® DNA Trace

1	**Lyse sample**		4–8 mL FLB 50 µL Proteinase K 56 °C, 1 h
2	**Clarify sample**		≥ 5,000 x *g*, 10 min
3	**Adjust DNA binding conditions**		3.5 mL ethanol Vortex
4	**Bind DNA**	 	Load sample 3,000 x *g*, 3 min
5	**Wash silica membrane**	 	1st wash 2.5 mL BW 2st wash 5 mL B5 3rd wash 5 mL B5 1st, 2nd, 3rd 3,000 x *g*, 3 min
6	**Dry silica membrane**		3,000 x *g*, 10 min
7	**Elute DNA**	 	100 µL BE (70 °C) RT, 2 min 3,000 x *g*, 3 min

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1 Components

1.1 Kit contents

	NucleoSpin® DNA Trace	
	4 preps	**25 preps**
REF	**740942.4**	**740942.25**
Lysis Buffer FLB	50 mL	250 mL
Wash Buffer BW	13 mL	75 mL
Wash Buffer B5 (Concentrate)*	12 mL	100 mL
Elution Buffer BE**	13 mL	13 mL
NucleoSpin® DNA Trace F Columns (plus Collection Tubes)	4	25
Proteinase K (lyophilized)*	6 mg	30 mg
Proteinase Buffer PB	1.8 mL	1.8 mL
Collection Tubes (50 mL)	4	25
Elution Tubes (0.5 mL)	4	25
User manual	1	1

* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- Ethanol (96–100 %) to prepare Buffer B5 and to adjust DNA binding conditions)

Consumables

- Disposable pipette tips
- 15 mL and 50 mL centrifugation tubes

Equipment

- Manual pipettors
- Centrifuge with swing-out rotor, suitable for 15 mL amd 50 mL tubes
- Suitable homogenization device (e.g., mortar and pestle, rotor-stator)
- Personal protection equipment (e.g., lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended that first-time users of the **NucleoSpin® DNA Trace** kit read the detailed protocol sections of this user manual. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

NucleoSpin® DNA Trace allows DNA isolation from cells, tissue, and many other sources. Lysis is achieved by incubation of homogenized samples in a solution containing chaotropic ions and Proteinase K. Appropriate conditions for binding of DNA to the silica membrane in the **NucleoSpin® DNA Trace F Columns** are created by chaotropic salt and ethanol. The binding process is reversible and specific to nucleic acids. Contaminations are removed by repeated washing with 2 different ethanolic buffers. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

2.2 Kit specifications

- **NucleoSpin® DNA Trace** kit is designed for the preparation of highly pure genomic DNA from small amounts of any tissue, cells and, forensic samples, for example dried blood spots. The **NucleoSpin® DNA Trace F Columns** included in the kit are ideally suited for collecting small amounts of nucleic acids from large volumes because these columns are shaped like a funnel combining a large volume capacity with a small diameter of the binding membrane (F means funnel). The DNA isolated by **NucleoSpin® DNA Trace F Columns** can be used directly for PCR or other enzymatic reactions.

- Age, storage conditions, quantity, and consistency of samples can affect DNA quality, and therefore the protocol may be adapted accordingly (e.g., increasing incubation time). For successful DNA preparation, it is essential that the sample is lysed well and separated afterwards – only clear lysates should be loaded onto **NucleoSpin® DNA Trace F Columns** in order to avoid clogging of the silica membrane.

- The **NucleoSpin® DNA Trace** kit allows purification of up to 20 µg of pure genomic DNA with an A_{260}/A_{280} ratio of between 1.70 and 1.90. Some samples (especially forensic samples) may contain only traces of DNA. However, the amount will be sufficient for amplification and detection reactions.

- Additional enzymes, which are not included in the kit, may be necessary for lysis of certain bacteria (e.g., lysozyme, lysostaphine).

- Support protocol for the isolation of genomic DNA from human bones. For this application additional Buffer T1, Buffer B3, and Proteinase K are necessary. Therefore MACHEREY-NAGEL offers the **NucleoSpin® DNA Trace Bone Buffer Set** (see ordering information). This buffer set is especially designed for completion of the **NucleoSpin® DNA Trace** kit. It is suited for 25 preparations of genomic DNA from human bones in conjunction with the **NucleoSpin® DNA Trace** kit (REF 740942.25).

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® DNA Trace
Technology	Silica-membrane technology
Format	Funnel columns
Sample material	Forensic samples, buccal swabs, blood spots
Sample size	Forensic samples which can be extracted with up to 8 mL Lysis Buffer FLB (in general 10 mg tissue, < 10^5 cells)
Fragment size	200 bp–approx. 50 kbp

Table 1: Kit specifications at a glance	
Typical recovery	> 70 % for amounts > 10 ng
A_{260}/A_{280}	1.7–1.9
Elution volume	100 µL
Preparation time	60 min/prep (without Proteinase K incubation time which needs > 1 h)
Binding capacity	20 µg

- **Forensic quality product:**

  NucleoSpin® DNA Trace is certified as forensic quality product. Consumables used in forensics need to be treated carefully to prevent DNA contamination. MACHEREY-NAGEL therefore has a stringently controlled production process to avoid DNA contamination of consumables. Further, MACHEREY-NAGEL uses ethylene oxide (EO) treatment to remove amplifiable DNA, which might still be introduced during the manufacturing process. MACHEREY-NAGEL products carrying the forensic quality seal, contain plastic materials that are EO treated. This means, DNA of any kind, which might still be introduced into plastic consumables during the production process, is inactivated by means of the treatment with ethylene oxide, in order to prevent the generation of accidental human profile by PCR amplification. Ethylene oxide treatment has been shown to be the method of choice to prevent DNA profiles due to DNA contamination. **(**Shaw *et al.***,** 2008).

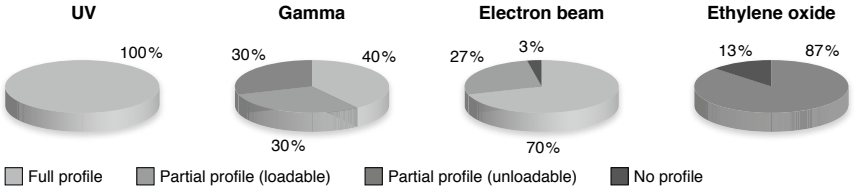


Figure 1: According to Shaw *et al.*, 2008, Comparison of the effects of sterilization techniques on subsequent DNA profiling. Int J Legal Med 122: 29-33.

3 Storage conditions and preparation of working solutions

Attention: Buffers FLB and BW contain chaotropic salts! Wear gloves and goggles!

CAUTION: Buffers FLB and BW contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- All kit components can be stored at room temperature (18–25 °C) and are stable for at least one year.
- Upon storage, especially at low temperatures, a white precipitate may form in Lysis Buffer FLB. Such precipitates have to be dissolved by incubating at 45–50 °C for 10 min before use.

Before starting any **NucleoSpin® DNA Trace** protocol, prepare the following:

- **Wash Buffer B5:** Add the indicated volume of ethanol (96–100 %) to **Buffer B5 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer B5 at room temperature (18–25 °C) for at least one year.
- Before first use of the kit, add the indicated volume (see table below or on the bottle) of Proteinase Buffer PB to dissolve lyophilized **Proteinase K**. Proteinase K solution is stable at - 20 C for at least 6 months.

	NucleoSpin® DNA Trace	
	4 preps	**25 preps**
REF	**740942.4**	**740942.25**
Wash Buffer B5 (Concentrate)	12 mL Add 48 mL ethanol	100 mL Add 400 mL ethanol
Proteinase K	6 mg Add 300 µL Proteinase Buffer	30 mg Add 1.5 mL Proteinase Buffer

4 Safety instructions

The following components of the **NucleoSpin® DNA Trace** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
FLB	Guanidine hydrochloride 1–10 % *Guanidinhydrochlorid 1–10 %*	Substance does not have to be specially labeled as hazardous *Die Substanz muss nicht als gefährlich gekennzeichnet werden.*		
BW	Guanidine hydrochloride 36–50 % + isopropanol 20–50 % *Guanidinhydrochlorid 36–50 % + Isopropanol 20–50 %*	Warning *Achtung*	226, 302, 319, 336	210, 233, 280, 301+312, 305+351+338, 330, 337+313, 403+235
Proteinase K	Proteinase K, lyophilized *Proteinase K, lyophilisiert*	Danger *Gefahr*	315, 319, 334, 335	261, 280, 302+352, 304+340, 305+351+338, 312, 332+313, 337+313, 342+311, 403+233

Hazard phrases

H 226	Flammable liquid and vapour. *Flüssigkeit und Dampf entzündbar.*
H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 315	Causes skin irritation. *Verursacht Hautreizungen.*
H 319	Causes serious eye irritation. *Verursacht schwere Augenreizung.*
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled. *Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.*
H 335	May cause respiratory irritation. *Kann die Atemwege reizen.*
H 336	May cause drowsiness or dizziness. *Kann Schläfrigkit und Benommenheit verursachen.*

Precaution phrases

P 210	Keep away from heat / sparks / open flames / hot surfaces – No smoking. *Von Hitze / Funken / offener Flamme / heißen Oberflächen fernhalten.*
P 233	Keep container tightly closed *Behälter dicht verschlossen halten.*
P 261	Avoid breathing dust. *Einatmen von Staub vermeiden.*
P 280	Wear protective gloves / eye protection. *Schutzhandschuhe / Augenschutz tragen.*
P 301+312	IF SWALLOWED: Call a POISON CENTER/ doctor/…/if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /… anrufen.*
P 302+352	IF ON SKIN: Wash with plenty of water/… *BEI KONTAKT MIT DER HAUT: Mit viel Wasser/… waschen.*
P 304+340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. *BEI EINATMEN: An die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.*
P 305+351+338	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. *BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter spülen.*
P 312	Call a POISON CENTER/ doctor/…/if you feel unwell. *Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /… anrufen.*
P 330	Rinse mouth. *Mund ausspülen.*
P 332+313	If skin irritation occurs: Get medical advice / attention. *Bei Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 337+313	Get medical advice / attention. *Bei anhaltender Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER/ doctor/… *Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM /Arzt/… anrufen.*
P 403+233	Store in a well ventilated place. Keep container tightly closed. *Behälter dicht verschlossen an einem gut belüfteten Ort aufbewahren.*
P 403+235	Store in a well ventilated place. Keep cool. *Kühl an einem gut belüfteten Ort aufbewahren.*

For further information please see Material Safety Data Sheets ***(www.mn-net.com).***

Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ***(www.mn-net.com)****.*

5 Protocols

5.1 Isolation of genomic DNA from solid samples, for example small amounts of cells or tissue (forensic samples)

Before starting the preparation:

- Check that Wash Buffer B5 was prepared according to section 3.
- Preheat Elution Buffer BE to 70 °C.

1 Lyse sample

Place the sample in a 15 mL centrifuge tube (not provided) and add **4–8 mL Buffer FLB**. The sample should be covered completely with Buffer FLB.

Solid samples should be homogenized by commercial tools (pestle and mortar, rotor-stator homogeniser). In general, 10 mg tissue, < 10^5 cells or any DNA-containing solid sample can be used. Forensic samples (dried blood spots, chewing gum, swabs, etc.) should be covered completely with lysis buffer.

+ 4–8 mL FLB

Add **50 µL Proteinase K** stock solution, mix by vortexing, and incubate at **56 °C** in a (shaking) water bath until complete lysis is obtained **(1–3 h or overnight)**.

Vortexing every 15 min (3–4 times) leads to shorter lysis times if no shaking water bath/incubator is available. Final incubation at 70–100 °C for 5 min may be recommended for optimal denaturation and lysis of difficult samples (e.g., dried, old or clotted blood samples).

+ 50 µL Proteinase K

56 °C, 1 h

2 Clarify sample

Afterwards, any insoluble particles remaining in the sample have to be removed by centrifugation for **10 min** at **≥ 5.000 x *g*** in order to avoid clogging of the NucleoSpin® DNA Trace membrane.

≥ 5,000 x *g*, 10 min

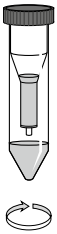
3 Adjust DNA binding conditions

Add **3.5 mL ethanol** (96–100 %) to **4 mL cleared FLB-lysate** and vortex the mixture. Use proportionally up scaled volumes of ethanol, if more FLB-lysate has been prepared in step 1.

+ 3.5 mL ethanol

Vortex

4 Bind DNA



Load sample

Pipette mixture onto the **NucleoSpin® DNA Trace F Column**.

3,000 x *g*, 3 min

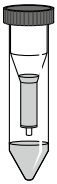
Centrifuge for **3 min** at **3,000 x *g***. Discard flow-through with Collection Tube. Put the NucleoSpin® DNA Trace F Column into a fresh Collection Tube (provided).

5 Wash silica membrane

+ 2.5 mL BW

1st wash

Add **2.5 mL Buffer BW** to the NucleoSpin® DNA Trace F Column. Centrifuge for **3 min** at **3,000 x *g***.



3,000 x *g*, 3 min

+ 5 mL B5

2nd wash

Add **5 mL Buffer B5** to the NucleoSpin® DNA Trace F Column. Centrifuge for **3 min** at **3,000 x *g***, discard flow-through and reuse Collection Tube.

3,000 x *g*, 3 min

+ 5 mL B5

3rd wash

Add **5 mL Buffer B5** to the NucleoSpin® DNA Trace F Column. Centrifuge for **3 min** at **3,000 x *g***, discard flow-through and reuse Collection Tube.

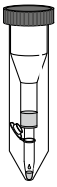
3,000 x *g*, 3 min

6 Dry silica membrane

3,000 x *g*, 10 min

Centrifuge additional **10 min** at **3,000 x *g*** in order to remove **Buffer B5** completely.

7 Elute DNA



+ 100 µL BE (70 °C)

RT, 2 min

Attach the supplied Elution Tube (0.5 mL) with adaptor to the NucleoSpin® DNA Trace F Column and insert assembly into a new 50 mL centrifufe tube (not provided). Pipette **100 µL Buffer BE** (preheated to 70 °C) onto the NucleoSpin® membrane and incubate for **2 min** at **room temperature**.

3,000 x *g*, 3 min

Centrifuge for **3 min** at **3,000 x *g*** to collect the nucleic acid-containing fraction.

Remove the elution tube containing the nucleic acids and keep it for further use.

5.2 Isolation of genomic DNA from human bones

Before starting with the preparation, please read remarks below.

- Before starting with the preparation, set incubators or water baths to 56 °C and 70 °C, respectively. Before elution, equilibrate Elution Buffer BE to 70 °C.

Attention:

- The list numbers in this support protocol do not correspond with the list numbers in section 5.1 and protocol-at-a-glance.
- Additional Buffer T1, Buffer B3 and Proteinase K is necessary. The **NucleoSpin® DNA Trace Bone Buffer Set** (REF 740943.25) is especially designed for completion of the NucleoSpin® DNA Trace kit. It is suited for 25 preparations of genomic DNA from human bones in conjunction with the NucleoSpin® DNA Trace kit (REF 740942.25).
- Preparation of **Lysis Buffer B3**: Transfer the total contents of **Buffer B1** to **Buffer B2** and mix well. The resulting Buffer B3 is stable for at least one year at room temperature.
- For each prep, 2 mL additional buffer is necessary (0.5 M EDTA/ 0.25 M PO_4^{3-}, pH 8, not included in the NucleoSpin® DNA Trace Bone Buffer Set).

1 Prepare sample

Mill 1 g bone to a fine powder.

2 Pre-lyse sample

Add **2 mL buffer** (0.5 M EDTA/0.25 M PO_4^{3-}, pH 8) and **7 mL Buffer T1** and **100 µL Proteinase K solution**. Vortex to mix. Be sure that the samples are completely covered with lysis solution.

If processing several samples, Proteinase K and Buffer T1 may be premixed directly before use. Do never mix Bufer T1 and Proteinase K more than 10–15 min before addition to the sample: Proteinase K tends to self-digestion in Buffer T1 without substrate.

Incubate at **56 °C overnight**.

Afterwards incubate sample for **48 h** at **4 °C** on a shaking incubator.

3 Lyse sample

Vortex the samples. Add **8 mL Buffer B3**, vortex vigorously and incubate at **70 °C** for **10 min**. Vortex briefly.

Centrifuge for **10 min** at **5,000 x *g*** and transfer the supernatant to a new microcentrifuge tube.

4 Adjust DNA binding conditions

Add **8.4 mL ethanol** (96–100 %) to the sample and vortex vigorously.

5 Bind DNA

For each sample, take one **NucleoSpin® DNA Trace F Column** placed in a Collection Tube (50 mL). Apply the sample successively to the column. Centrifuge for **3 min** at **3,000 x *g***. Discard the flow-through and place the column back into the Collection Tube.

6 Wash silica membrane

1st wash

Add **3 mL Buffer BW**. Centrifuge for **3 min** at **3,000 x *g***. Discard the flow-through and place the column back into the Collection Tube.

2nd wash

Add **3 mL Buffer B5** to the column and centrifuge for **3 min** at **3,000 x *g***. Discard the flow-through and place the column back into the Collection Tube.

3rd wash

Add **3 mL Buffer B5** to the column and centrifuge for **3 min** at **3,000 x *g***. Discard the flow-through and place the column back into the Collection Tube.

7 Dry silica membrane

Centrifuge the column for **10 min** at **3,000 x *g***.

Residual ethanol is removed during this step.

8 Elute highly pure DNA

Attach the supplied Elution Tube with adaptor to the NucleoSpin® DNA Trace F Column and insert assembly into a new 50 mL centrifuge tube (not provided). Add **60 µL Buffer BE** (preheated to 70 °C). Incubate at **room temperature** for **2 min**.

Centrifuge for **3 min** at **3,000 x *g*** to collect the nucleic acid-containing fraction.

Remove the elution tube containing the nucleic acids and keep it for further use.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or poor DNA yield, poor DNA quality	*Incomplete sample lysis* • Sample was not thoroughly homogenized and mixed with Buffer FLB/ Proteinase K. The mixture has to be shaken continuously. Alternatively, prolong incubation time with Proteinase K. *Reagents not applied properly* • Prepare Buffer B5 and Proteinase K solutions according to instructions (section 3). Add ethanol to lysates before loading them on NucleoSpin® DNA Trace F Columns. *Suboptimal elution of DNA from the column* • Apply Elution Buffer BE (70 °C) directly onto the center of the silica membrane and incubate for 2 min. Elution efficiencies decrease dramatically, if elution is done with other buffers at pH ≤ 7.0.
Poor DNA qualty and/or suboptimal performance of genomic DNA in enzymatic reactions	*RNA in sample* • If RNA-free DNA is desired, add 20 µL of RNase A solution (20 mg/mL) to Lysis Buffer FLB. *Carry-over of ethanol* • Be certain to centrifuge ≥ 5 min at 3,000 x *g* in order to remove all of ethanolic Buffer B5 before eluting the DNA. If for any reason, the level of Buffer B5 has reached the column outlet after the second wash, discard flow-through. Place the NucleoSpin® DNA Trace F Column back into the Collection Tube, and centrifuge again.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® DNA Trace	740942.4/.25	4/25 preps
NucleoSpin® Funnel Column	740959	30 columns
Buffer FLB	740322.500	500 mL
Buffer BW	740922	100 mL
Buffer B5 Concentrate (for 100 mL Buffer B5)	740921	20
NucleoSpin® DNA Trace Bone Buffer Set	740943.25	1 set
Proteinase K	740506	100 mg
RNase A	740505.50 740505	50 mg 100 mg
NucleoSpin® Forensic Filters	740988.10/.50/.250	10/50/250 pieces
NucleoSpin® Forensic Filters (Bulk)	740988.50B/.250B/1000B	50/250/1000 pieces

Visit ***www.mn-net.com*** for more detailed product information.

6.3 Product use restriction/warranty

NucleoSpin® DNA Trace kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN VITRO*-diagnostic use. Please pay attention to the package of the product. *IN VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

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